Kam 09/916,250

=> d his 1

(FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 09:52:56 ON 07 MAY 2003)
L12 42 DUP REM L11 (31 DUPLICATES REMOVED)

=> d que l12
L1 634 SEA TAKAGAKI K?/AU
L2 99 SEA L1 AND PROTEOGLYCAN
L3 16 SEA L2 AND CARTILAG?
L4 81199 SEA PROTEOGLYCAN#
L5 5790 SEA (PURIF? OR EXTRACT? OR ISOLAT?)(5A) L4
L6 13 SEA L5 AND ACETIC(A) ACID
L7 60 SEA L5 AND (ETHANOL OR ETHYL(A) ALCOHOL)
L8 21 SEA L7 AND CARTILAG?
L9 33 SEA L5 AND ACID? AND (ETHANOL OR ETHYL(A) ALCOHOL)
L10 1 SEA L5 AND (ETHANOL OR ETHYL(A) ALCOHOL) AND ((SODIUM OR NA)(A) CHLORIDE)
L11 73 SEA L3 OR L6 OR (L8 OR L9 OR L10)
L12 42 DUP REM L11 (31 DUPLICATES REMOVED)

=> d ibib abs l12 1-42

L12 ANSWER 1 OF 42 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:157148 HCAPLUS
DOCUMENT NUMBER: 136:163703
TITLE: A method for **extraction** and **purification** of **cartilage** type **proteoglycan**
INVENTOR(S): **Takagaki, Keeichi**
PATENT ASSIGNEE(S): Kakuhiro Co., Ltd., Japan
SOURCE: Eur. Pat. Appl., 8 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1182209	A2	20020227	EP 2001-117771	20010801
EP 1182209	A3	20030205		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002069097	A2	20020308	JP 2000-251071	20000822
US 2002045735	A1	20020418	US 2001-916250	20010730

PRIORITY APPLN. INFO.: JP 2000-251071 A 20000822
AB The present invention relates to a new method for extn. and **purifn**. of **cartilage** type **proteoglycan**, and is to provide a method for extn. of crude **proteoglycan** characterized by the use of **acid** as eluting solvent of **cartilage**.

L12 ANSWER 2 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2002:493607 SCISEARCH
THE GENUINE ARTICLE: 558PM
TITLE: Cleavage of the xylosyl serine linkage between a core peptide and a glycosaminoglycan chain by cellulases
AUTHOR: **Takagaki K**; Iwafune M; Kakizaki I; Ishido K;

Search completed by David Schreiber 308-4292

Kato Y; Endo M (Reprint)
CORPORATE SOURCE: Hirosaki Univ, Sch Med, Dept Biochem, 5 Zaifu Cho, Hirosaki, Aomori 0368562, Japan (Reprint); Hirosaki Univ, Sch Med, Dept Biochem, Hirosaki, Aomori 0368562, Japan; Hirosaki Univ, Fac Educ, Food Sci Lab, Hirosaki, Aomori 0368560, Japan
COUNTRY OF AUTHOR: Japan
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (24 MAY 2002) Vol. 277, No. 21, pp. 18397-18403.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 46

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We previously found that endo-beta-xylosidase from Patinopecten is an endo-type glycosidase that cleaves the xylosyl serine linkage between a glycosaminoglycan chain and its core protein (Takagaki, K., Kon, A., Kawasaki, H., Nakamura, T., Tamura, S., and Endo, M. (1990) J. Biol. Chem. 265, 854-860). Screening for endo-beta-xylosidase activity in several cellulases detected this activity in the enzymes from Aspergillus niger, Penicillium funiculosum, Trichoderma reesei, Trichoderma viride, and Irpex lacteus. The cellulase derived from A. niger was purified, and its molecular weight was determined to be 26,000 by SDS-PAGE. Examination of the specificity of the cellulase revealed that 1) the enzyme acts on the linkage region (xylosyl serine) between a core peptide and a glycosaminoglycan chain; 2) enzymatic activity is greater with shorter glycosaminoglycan chains; 3) the enzyme readily hydrolyzes the linkage in glycosaminoglycan peptides, but intact **proteoglycan** is cleaved only slowly; and 4) the activity is unaffected by the glycosaminoglyean component (chondroitin sulfate, dermatan sulfate, and heparan sulfate). Judging from these enzymatic characteristics, this cellulase is different from the endo-beta-xylosidase of Patinopecten. We believe that this cellulase will become a useful tool in the further development of glycotechnology, because, like the endo-beta-xylosidase of Patinopecten, it enables the release of intact glycosaminoglyeans from glycosaminoglycan peptides.

L12 ANSWER 3 OF 42 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002192400 MEDLINE
DOCUMENT NUMBER: 21922830 PubMed ID: 11805117
TITLE: Enzymatic attachment of glycosaminoglycan chain to peptide using the sugar chain transfer reaction with endo-beta-xylosidase.
AUTHOR: Ishido Keinosuke; **Takagaki Keiichi**; Iwafune Mito; Yoshihara Syuichi; Sasaki Mutsuo; Endo Masahiko
CORPORATE SOURCE: Department of Biochemistry and Second Department of Surgery, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Apr 5) 277 (14) 11889-95.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020403

Search completed by David Schreiber 308-4292

Last Updated on STN: 20030105
Entered Medline: 20020513

AB Endo-beta-xylosidase from the mid-gut gland of the molluscus Patinopecten is an endo-type glycosidase that hydrolyzes the xylosyl serine linkage between a core protein and a glycosaminoglycan (GAG) chain, releasing the intact GAG chain from **proteoglycan**. In this study, we investigated GAG chain transfer activity of this enzyme, in order to develop a method for attaching GAG chains to peptide. Peptidochondroitin sulfate (molecular mass of sugar chain, 30 kDa) from bovine tracheal **cartilage** as a donor and butyloxycarbonyl-leucyl-seryl-threonyl-arginine-(4-methylcoumaryl-7-amide) as an acceptor were incubated with endo-beta-xylosidase. As a result, a reaction product with the same fluorescence as the acceptor peptide was observed. High pressure liquid chromatography analysis, cellulose acetate membrane electrophoresis, and enzymatic digestion showed that this reaction product had the chondroitin sulfate (ChS) from the donor. Furthermore, the acceptor peptide was released from this reaction product after hydrolysis by endo-beta-xylosidase. Therefore, it was confirmed that the ChS chain released from the donor was transferred to the acceptor peptide by the GAG chain transfer reaction of endo-beta-xylosidase. The optimal pH for hydrolysis by this enzyme was found to be about 4.0, whereas that for this reaction was about 3.0. Not only the ChS but also the dermatan sulfate and the heparan sulfate were transferred to the acceptor peptide by this reaction. By using this reaction, the GAG chain could be attached to the peptide in one step. The GAG chain transfer reaction of endo-beta-xylosidase should be a significant glycotechnological tool for the artificial synthesis of **proteoglycan**.

L12 ANSWER 4 OF 42 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2002408377 MEDLINE
DOCUMENT NUMBER: 22151995 PubMed ID: 12162349
TITLE: Chicken keel **cartilage** as a source of chondroitin sulfate.
AUTHOR: Luo X M; Fosmire G J; Leach R M Jr
CORPORATE SOURCE: Department of Nutrition, The Pennsylvania State University, University Park 16802, USA.
SOURCE: POULTRY SCIENCE, (2002 Jul) 81 (7) 1086-9.
Journal code: 0401150. ISSN: 0032-5791.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20020807
Last Updated on STN: 20030125
Entered Medline: 20030124

AB Chondroitin sulfate is used extensively as a treatment for osteoarthritis. This study was conducted to evaluate whether chondroitin sulfate could be isolated from chicken keel **cartilage** in sufficient quantities and of requisite quality to make it a feasible source of chondroitin sulfate. **Proteoglycans** were **extracted** from chicken keel **cartilage** obtained immediately after slaughter by using 3 M MgCl2 at room temperature. The extract was then dialyzed and digested with papain to remove proteins. Glycosaminoglycans were obtained by **ethanol** precipitation, lyophilized, and characterized by using gel filtration on Sepharose CL-6B columns. Guanidine-HCI extraction was also used as a control to investigate the efficiency of extraction using MgCl2. Results showed that, from every gram of wet or non-lyophilized keel **cartilage**, 32.9 +/- 4.8 mg (dry weight) of glycosaminoglycans

could be obtained following MgCl2 extraction. Analyses revealed that 75.5 +/- 4.2% of these glycosaminoglycans were chondroitin sulfate. Chromatographic analyses showed a single symmetrical peak, which could be almost entirely removed by prior digestion with chondroitinase ABC, indicating that the material in the peak was in fact chondroitin sulfate. The average molecular weight (also called relative molecular mass, Mr) of the glycosaminoglycans was also estimated (Mr 48,500). Characterization using polyacrylamide or agarose gel electrophoresis showed diffuse bands containing chondroitin sulfate, which could be entirely removed by prior digestion with chondroitinase ABC. This study shows that chicken keel **cartilage** is a readily available source of chondroitin sulfate.

L12 ANSWER 5 OF 42 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:262874 BIOSIS
DOCUMENT NUMBER: PREV200100262874
TITLE: Avian keel **cartilage** as a source of chondroitin sulfate.
AUTHOR(S): Luo, Xin (1); Fosmire, Gary J. (1); Leach, Roland M., Jr. (1)
CORPORATE SOURCE: (1) Pennsylvania State University, University Park, PA USA
SOURCE: FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A993. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Chondroitin sulfate, prepared from bovine tracheal **cartilage** or shark **cartilage**, is used extensively as a treatment for osteoarthritis in both the United States and Europe. This study was conducted to evaluate whether chondroitin sulfate could be isolated from avian keel **cartilage** in sufficient quantities and of requisite quality to make it a feasible source for commercial use. **Proteoglycans** were **extracted** from chicken keel **cartilage** by using 3M MgCl2 at room temperature, dialyzed, and digested with papain to remove proteins. After **ethanol** precipitation glycosaminoglycans obtained by lyophilization were characterized by using gel filtration on Sepharose CL-6B column. Analyses of chromatographic fractions showed a single symmetrical peak, which was almost entirely removed by digestion with chondroitinase ABC before applying to the column. This indicates that much of the material in the peak was in fact chondroitin sulfate. From every gram of "wet" or non-lyophilized keel **cartilage**, 38.9 +- 2.4 mg (dry weight) of glycosaminoglycans were obtained. Analyses revealed that 74.8% +- 17.3% of these glycosaminoglycans were chondroitin sulfate. Thus, avian keel **cartilage** appears to be a feasible source of commercially used chondroitin sulfate.

L12 ANSWER 6 OF 42 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:490169 HCAPLUS
DOCUMENT NUMBER: 137:277009
TITLE: Effect of **proteoglycan** on experimental colitis
AUTHOR(S): Majima, Mitsuo; **Takagaki, Keiichi**; Sudo, Shin-ichiro; Yoshihara, Syuichi; Kudo, Yoshiaki; Yamagishi, Shohei
CORPORATE SOURCE: Kakuhiro Co. Ltd., Aomori, 030-8543, Japan

Search completed by David Schreiber 308-4292

SOURCE: International Congress Series (2001), 1223(New Developments in Glycomedicine), 221-224
CODEN: EXMDA4; ISSN: 0531-5131
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The effect of **proteoglycan** (PG) on colitis was examd. in animal expts. using mice. The PG used was extd. from nasal **cartilage** of salmon head with 4% acetic acid and prepd. by pptn. with ethanol followed by dialysis. The PG contained about 7% protein, and had a mol. mass of 344 kDa on SDS/PAGE. The glycosaminoglycan (GAG) sugar chains of the PG were composed of hexosamine, uronic acid and sulfate at a molar ratio of 1.0:1.0:0.7. The mice were divided into a control group and an administration group. The control group was given free access to drinking water contg. dextran sulfate sodium salt (DSS) to induce colitis. On the other hand, the administration group was given free access to drinking water contg. DSS and PG. Then, the time course of survival rates in both groups were measured. In the administration group, the survival rate increased significantly in comparison with that of the control group. The difference in the survival rates indicated that the onset of mouse colitis induced by DSS was inhibited by administration of the PG.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 2001:898373 SCISEARCH
THE GENUINE ARTICLE: 488VQ

TITLE: Structural varieties of small proteoglycans in human spinal ligament

AUTHOR: Yukawa M; **Takagaki K**; Itabashi T; Ueyama K; Harata S; Endo M (Reprint)
CORPORATE SOURCE: Hirosaki Univ, Sch Med, Dept Biochem, 5 Zaifu Cho, Hirosaki, Aomori 0368562, Japan (Reprint); Hirosaki Univ, Sch Med, Dept Biochem, Hirosaki, Aomori 0368562, Japan; Hirosaki Univ, Sch Med, Dept Orthopaed Surg, Hirosaki, Aomori 0368562, Japan
COUNTRY OF AUTHOR: Japan

SOURCE: CONNECTIVE TISSUE RESEARCH, (MAR 2001) Vol. 42, No. 3, pp. 209-222.
Publisher: GORDON BREACH PUBLISHING, TAYLOR & FRANCIS GROUP, 325 CHESTNUT ST, 8TH FL, PHILADELPHIA, PA 19106 USA

ISSN: 0300-8207.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 41

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Three types of small **proteoglycan** were purified from human spinal ligaments by ultracentrifugation, ion-exchange chromatography, gel-chromatography, and hydrophobic chromatography. Two of them were identified as decorin and biglycan, and the other was thought to be a decorin-subtype. Molecular sizes of decorin and decorin-subtype were both 85 kDa, and that of biglycan was 200 kDa. N-Terminal amino acid sequence of decorin-subtype corresponded with that of decorin, although it was different from decorin in terms of composition of amino acids and glycosaminoglycan chains, and reactivity with anti-human decorin antibody. The ratios of chondroitin sulfate to dermatan sulfate contained in the three proteoglycans were different, and the location of that in glycosaminoglycan chains was also thought to be different. It was

demonstrated that three types of **proteoglycan** which are structurally different are present in extracellular matrix.

L12 ANSWER 8 OF 42 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:151546 BIOSIS
DOCUMENT NUMBER: PREV200200151546
TITLE: Physical and enzymatic perturbation of the architecture of the tunica media destroys its inherent thromboresistance.
AUTHOR(S): Komorowicz, Erzsebet; McBane, Robert D. (1); Fass, David N. (1)
CORPORATE SOURCE: (1) Internal Medicine, Mayo Clinic, Rochester, MN USA
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 2, pp. 55b. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 2 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Background: Perfused with anticoagulated whole blood at high shear, the tunica media, in situ, binds 5% or less the number of platelets than does the adventitia. There is a similar discrepancy in Willebrand factor (vWf) binding between the two vessel wall layers. We propose that there exists a chemical difference between the collagens of the media and the adventitia. This contrast may be a function intrinsic to the type of tissue specific collagen or result from accessory molecules complexed to the collagens in the tissue matrix. Methods: To examine the characteristics of the individual layers, they were physically torn from the external elastic lamina, frozen and sectioned and each examined for platelet and vWf binding. Sections were mounted in a parallel plate flow chamber and perfused with citrated whole blood at 3350/sec shear rate to measure platelet adhesion. Sections were also incubated with porcine vWf-coated beads to evaluate vWf binding sites. Alterations in matrix architecture were accomplished by subjecting porcine arteries to balloon catheterization or by treating cross sections with solvents, proteases or glucosaminoglycan lyases prior to flow chamber analysis. Results: When stripped from a muscular artery, the separated media shows marked enhanced binding of platelets at high shear and of vWf coated beads. When similar stretching is induced by ballooning a vessel, a 10 fold increase in both platelet and vWf-coated particle adherence is seen in the media while insignificant differences are seen in binding to the adventitia between normal and ballooned segments. Brief (30 min) exposure of vessel cross-sections to 0.5 M **acetic acid** or enzymes in the 10-30 nM concentration range promoted a 10-20 fold increase in platelet and specific particle binding to the media. Effective enzymes included, trypsin, chymotrypsin, plasmin, chondroitinase ABC and chondroitinase AC. Less effective at these doses was proteinase 3, and heparinase was ineffective up to 1 U/ml. Conclusions: The morphology of mature collagen fibrils has been shown to be influenced by proteoglyans which have specific geometric docking arrangements with the collagen bundles. We conclude that in the media, these proteoglycans hinder the binding of vWf to the collagen. Dimensional alterations disturb this interaction and restore vWf binding to the collagen allowing platelet adhesion at high shear. We also suggest that the **proteoglycan** is **extracted** in weak acid and is released when either the protein core or polysaccharide branches are cleaved enzymatically, thereby ablating the natural antithrombotic nature of the media.

L12 ANSWER 9 OF 42 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

Kam 09/916,250

ACCESSION NUMBER: 2001276200 EMBASE
TITLE: Biosynthesis of glycosaminoglycans and proteoglycans by the lymph node.
AUTHOR: Brown T.J.; Kimpton W.G.; Fraser J.R.E.
CORPORATE SOURCE: Dr. T.J. Brown, Department of Biochemistry, Monash University, Clayton 3168, Australia.
Tracy.Brown@med.monash.edu.au
SOURCE: Glycoconjugate Journal, (2000) 17/11 (795-805).
Refs: 33
ISSN: 0282-0080 CODEN: GLJOEW
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Previous studies of hyaluronan uptake and catabolism by lymph nodes indicated that the nodes might also add some HA of low molecular weight to the unabsorbed fraction that passes through from afferent to efferent lymph vessels. The ability of lymph nodes to synthesise HA and proteoglycans was therefore examined (i) by perfusion of [(3)H] acetate through an afferent lymph vessel in vivo, and recovery of labeled products from the efferent lymph vessel and from the node after perfusion; and (ii) by tissue culture of lymph nodes with [(3)H] acetate. Perfusion of lymph nodes with [(3)H] acetate in situ yielded: (a), in outflowing lymph, small amounts of chondroitin/dermatan sulfate within the first hour which continued to be produced for up to 24 h; heparin in the second hour and HA in the third. In the nodes removed 17 to 19 h later, equal amounts of hyaluronan and chondroitin/dermatan sulfate and heparan sulfate proteoglycans were detected. In the tissue culture of lymph nodes: (1) HA, heparin and proteoglycans of heparan sulfate and chondroitin/dermatan sulfate were released into the medium but in the cell **extract** only heparan sulfate **proteoglycan** was detected; and (ii) molecular weight of the released hyaluronan ranged widely but was mostly less than 4-5 x 10(5) D; heparan sulfate proteoglycan was 2.8 x 10(4) to 9.4 x 10(5) D; heparin 7.9 x 10(4) D and chondroitin sulfate 1.3 x 10(4) D, suggesting that the chondrotin sulfate were released from their proteoglycans core by enzymic degradation. It is concluded that lymph nodes can release HA, heparin, heparan sulfate and chondroitin/dermatan sulfate proteoglycans into efferent lymph but the amount of hyaluronan is likely to be small without immune or other stimulation and its molecular weight is lower than in other tissues.

L12 ANSWER 10 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1999:815804 SCISEARCH
THE GENUINE ARTICLE: 240DF
TITLE: Interaction between collagens and glycosaminoglycans investigated using a surface plasmon resonance biosensor
AUTHOR: Munakata H; **Takagaki K**; Majima M; Endo M
(Reprint)
CORPORATE SOURCE: HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, 5 ZAIFU CHO, HIROSAKI, AOMORI 0368562, JAPAN (Reprint); HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, HIROSAKI, AOMORI 0368562, JAPAN; KUSHIRO JR COLL, KUSHIRO 0850814, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: GLYCOBIOLOGY, (OCT 1999) Vol. 9, No. 10, pp. 1023-1027.
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0959-6658.

Search completed by David Schreiber 308-4292

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 31

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The interactions of glycosaminoglycans with collagens and other glycoproteins in extracellular matrix play important roles in cell adhesion and extracellular matrix assembly. In order to clarify the chemical bases for these interactions, glycosaminoglycan solutions were injected onto sensor surfaces on which collagens, fibronectin, laminin, and vitronectin were immobilized. Heparin bound to type V collagen, type IX collagen, fibronectin, laminin, and vitronectin; and chondroitin sulfate E bound to type II, type V, and type Vn collagen. Heparin showed a higher affinity for type IX collagen than for type V collagen. On the other hand, chondroitin sulfate E showed the highest affinity for type V collagen. The binding of chondroitin sulfate E to type V collagen showed higher affinity than that of heparin to type V collagen. These data suggest that a novel characteristic sequence included in chondroitin sulfate E is involved in binding to type V collagen.

L12 ANSWER 11 OF 42 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 1999:642114 HCAPLUS
DOCUMENT NUMBER: 132:131988
TITLE: Anti-complementary properties of polysaccharides isolated from fruit bodies of mushroom Pleurotus ostreatus
AUTHOR(S): Mee-Hyang, Kweon; Jang, Hyo; Lim, Wang-Jin; Chang, Hyo-Ihl; Kim, Chan-Wha; Yang, Han-Chul; Hwang, Han-Joon; Sung, Ha-Chin
CORPORATE SOURCE: Institute of Biotechnology, Graduate School of Biotechnology, Korea University, Seoul, 136-701, S. Korea
SOURCE: Journal of Microbiology and Biotechnology (1999), 9(4), 450-456
CODEN: JOMBES; ISSN: 1017-7825
PUBLISHER: Korean Society for Applied Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A high mol.-wt. water-sol. fraction (PO) obtained by the **ethanol** pptn. of 0.1 N NaOH exts. of the mushroom Pleurotus ostreatus showed 82% anti-complementary activity for complement consumption hemolysis. The PO consisted of 42% carbohydrate (wt./wt.), 50% protein (wt./wt.), and 3% uronic **acid** (wt./wt.). Fifty-eight percent of the anti-complementary activity decreased by periodate oxidn. and 22% by protease digestion, suggesting that the sugar and protein moieties are essential for this activity. Two polysaccharide fractions, PO-IIIa-1 and PO-IIIa-2, with anti-complementary activity were isolated from the PO using DEAE-Sepharose FF followed by Sephadex G-75 and Sepharose CL-6B gel permeation chromatogs. The PO-IIIa-2 was found by HPLC to be nearly homogeneous, with the mol. mass of 531 kDa, and showed 96% ITCH50 (inhibition against the total complement hemolysis of deionized water as the control) at a concn. of 1 mg/mL. This fraction contained galactose, mannose, fucose, and glucose with molar ratios of 1.75:1:0.65 and 0.59, resp. The majority of galactose and mannose units in the PO-IIIa-2 were composed of TGalp1.fwdarw., .fwdarw.6Galp1.fwdarw., .fwdarw.2,6Galp1.fwdarw., and .fwdarw.Manp1.fwdarw.. The PO-IIIa-1 (mol. mass of 2000 kDa), exhibiting higher activity than the PO-IIIa-2, was further **purified** into two fractions, unbound **proteoglycan** (PO-IIIa-1A) and bound glucomannan (PO-IIIa-1B), by

affinity chromatog. using ConA-Sepharose CL-4B. The anti-complementary activity of each affinity purified fraction decreased as compared to that of the native PO-IIIa-1 fraction, indicating that the formation of complex between both polysaccharide fractions was necessary for full anti-complementary activity.
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 12 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1998:6683 SCISEARCH
THE GENUINE ARTICLE: YL149
TITLE: Effect of monensin on the synthesis of beta-D-xyloside-initiated glycosaminoglycan and its linkage region oligosaccharides in human skin fibroblasts
AUTHOR: **Takagaki K**; Tazawa T; Munakata H; Nakamura T; Endo M (Reprint)
CORPORATE SOURCE: HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, 5 ZAIFU CHO, HIROSAKI, AOMORI 036, JAPAN (Reprint); HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, HIROSAKI, AOMORI 036, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF BIOCHEMISTRY, (DEC 1997) Vol. 122, No. 6, pp. 1129-1132.
Publisher: JAPANESE BIOCHEMICAL SOC, ISHIKAWA BLDG-3F, 25-16 HONGO-5-CHOME, BUNKYO-KU, TOKYO 113, JAPAN.
ISSN: 0021-924X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 18
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human skin fibroblasts were cultured with a fluorogenic xyloside, 4-methylumbelliferyl beta-D-xyloside (Xyl-MU) as an initiator, and the effects of monensin, which destroys the normal structure of the Golgi complex, on the synthesis of Xyl-MU-initiated glycosaminoglycan (GAG-MU) and its linkage region oligosaccharides were investigated, When the cells were incubated with Xy I-MU in the presence of monensin, the synthesis of GAG-MU was inhibited, In addition, the synthesis of Gal beta 1-3Gal beta 1-4Xyl beta 1-MU as an intermediate of GAG-MU was inhibited, whereas the synthesis of Gal beta 1-4Xyl beta 1-MU, which is formed prior to Gal beta 1-3Gal beta 1-4Xyl beta 1-MU, was not, These results indicate that inhibition of GAG-MU synthesis by monensin occurs at the point where the second galactose is joined to Gal beta 1-4Xyl beta 1-MU.

L12 ANSWER 13 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 96:307193 SCISEARCH
THE GENUINE ARTICLE: UE816
TITLE: CHARACTERIZATION AND BIOLOGICAL SIGNIFICANCE OF SIALYL ALPHA-2-3GALACTOSYL-BETA-1-4XYLOSYL-BETA-1-(4-METHYLUMBELLIFERONE) SYNTHESIZED IN CULTURED HUMAN SKIN FIBROBLASTS
AUTHOR: **TAKAGAKI K**; NAKAMURA T; SHIBATA S; HIGUCHI T; ENDO M (Reprint)
CORPORATE SOURCE: HIROSAKI UNIV, SCH MED, DEPT BIOCHEM 2, 5 ZAIFU CHO, HIROSAKI, AOMORI 036, JAPAN (Reprint); HIROSAKI UNIV, SCH MED, DEPT BIOCHEM 2, HIROSAKI, AOMORI 036, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF BIOCHEMISTRY, (APR 1996) Vol. 119, No. 4, pp. 697-702.
ISSN: 0021-924X.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 24

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human skin fibroblasts were incubated in the presence of a fluorogenic xyloside, 4-methyl-umbelliferyl-beta-D-xyloside (Xyl-MU), then the cultured medium was recovered, concentrated with a lyophilizer, and dialyzed against distilled water. The structures of the Xyl-MU derivatives purified from the dialyzable fraction were investigated. In addition to established glycosaminoglycans-MU (GAGs-MU), Gal-Gal-Xyl-MU, Gal-Xyl-MU, sulphate-GlcA-Xyl-MU, GlcA-Xyl-MU, and Xyl-Xyl-MU, which were induced by Xyl-MU, an oligosaccharide having fluorescence was purified using a combination of gel filtration, ion-exchange chromatography and high-performance liquid chromatography, then subjected to carbohydrate composition analysis, enzyme digestion, Smith degradation, H-1-NMR, and ion-spray mass spectrometric analysis. From the data obtained, the oligosaccharide was considered to have the structure SA alpha 2-3Gal beta 1-4Xyl beta 1-MU. The amount of MU-oligosaccharide in the cell culture increased with time and was dependent on the amount of Xyl-MU added. Its production was also different from that of Gal-Gal-Xyl-MU and Gal-Xyl-MU, which are biosynthetic intermediates of GAG-MU. Addition of CDP, an inhibitor of sialyltransferase, to the cell culture medium increased the secretion of GAG-MU. These results suggest that SA-Gal-Xyl-MU production may be related to the regulation of GAG-MU biosynthesis.

L12 ANSWER 14 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 97:8598 SCISEARCH
THE GENUINE ARTICLE: VX989
TITLE: Evidence for the presence of a large keratan sulphate proteoglycan in the human uterine cervix
AUTHOR: Fischer D C; Henning A; Winkler M; Rath W; Haubeck H D (Reprint); Greiling H
CORPORATE SOURCE: RHEIN WESTFAL TH AACHEN, INST KLIN CHEM & PATHOBIOCHEM, PAUWELSSTR 30, D-52057 AACHEN, GERMANY (Reprint); RHEIN WESTFAL TH AACHEN, INST KLIN CHEM & PATHOBIOCHEM, D-52057 AACHEN, GERMANY; RHEIN WESTFAL TH AACHEN, KLIN FRAUENHEILKUNDE & GEBURTSHILFE, D-52057 AACHEN, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: BIOCHEMICAL JOURNAL, (1 DEC 1996) Vol. 320, Part 2, pp. 393-399.
Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON, ENGLAND W1N 3AJ.
ISSN: 0264-6021.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 30

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Profound changes occur in the uterine cervix during pregnancy. In particular, the extracellular matrix of the connective tissue is remodelled extensively. To elucidate the mechanisms involved in this process, we have analysed the proteoglycan pattern in the human cervix from pregnant and non-pregnant women. Proteoglycans of the cervix tissue specimen were extracted with 4 M guanidine hydrochloride and precipitated with 80% **ethanol**. **Purification** of **proteoglycans** was performed by several chromatographic steps. Characterization of proteoglycans was done by SDS/PAGE before and after digestion with glycosaminoglycan-specific enzymes. Proteoglycans were

detected by combined Alcian Blue/silver staining or, after blotting of biotin-labelled proteoglycans on to poly(vinylidene difluoride) membrane, with peroxidase-conjugated avidin or by the use of keratan sulphate- or decorin-specific monoclonal antibodies. In contrast with previous reports, where only chondroitin/dermatan sulphate proteoglycans have been found in the uterine cervix, we have shown in the present study the existence of a large keratan sulphate proteoglycan with an M(r) > 220000 in cervix samples from non-pregnant and pregnant women. This proteoglycan showed a strong reaction with the keratan sulphate-specific monoclonal antibody 5D4 and could be degraded by keratanases. The size of the core protein of this keratan sulphate proteoglycan was estimated to be about M(r) 220000.

L12 ANSWER 15 OF 42 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
ACCESSION NUMBER: 1996:388688 HCAPLUS
DOCUMENT NUMBER: 125:52677
TITLE: Effect of aqueous and nonaqueous fixatives on the quantitative estimation of collagen-proteoglycan interaction in tissue sections
AUTHOR(S): Toledo, Olga Maria S.; Taniwaki, Noemi N.; Saldiva, Paulo H. N.; Montes, Gregorio S.
CORPORATE SOURCE: School Medicine, University Sao Paulo, Sao Paulo, 01246-903, Brazil
SOURCE: Biotechnic & Histochemistry (1996), 71(3), 109-114
CODEN: BIHIEU; ISSN: 1052-0295
PUBLISHER: Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The present study was designed to assess the influence of aq. and nonaq. fixatives on the quant. estn. of collagen-proteoglycan interaction in tissue sections. Tissues contg. different collagen types and distinct sulfated **proteoglycan** classes were **isolated** from pig costal **cartilage**, human skin, and the inner muscular layer of the dog small intestine and fixed using aq. or nonaq. methods. The results showed that the best fixation method was exposure to paraformaldehyde gas. When using aq. fixatives, proteoglycans were lost to different degrees among the various tissues analyzed, reflecting differences in chem. properties of proteoglycan classes and/or in their interactions with other matrix components such as collagen.

L12 ANSWER 16 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 95:236571 SCISEARCH
THE GENUINE ARTICLE: QM952
TITLE: HYALURONIC-ACID-DEFICIENT EXTRACELLULAR-MATRIX INDUCED BY ADDITION OF 4-METHYLUMBELLIFERONE TO THE MEDIUM OF CULTURED HUMAN SKIN FIBROBLASTS
AUTHOR: NAKAMURA T; **TAKAGAKI K**; SHIBATA S; TANAKA K; HIGUCHI T; ENDO M (Reprint)
CORPORATE SOURCE: HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, 5 ZAIFU CHO, HIROSAKI, AOMORI 036, JAPAN (Reprint); HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, HIROSAKI, AOMORI 036, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (17 MAR 1995) Vol. 208, No. 2, pp. 470-475.
ISSN: 0006-291X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 27
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

Search completed by David Schreiber 308-4292

AB The effects of xylosyl-beta-D-(4-methylumbelliferone) and its aglycone, 4-methylumbelliferone, on hyaluronic acid synthesis were investigated in cultured human skin fibroblasts. Xylosyl-beta-D-(4-methylumbelliferone) added to the medium of cultured cell reduced the synthesis of hyaluronic acid. Furthermore, 4-methylumbelliferone reduced the production of hyaluronic acid markedly. In addition, 4-methylumbelliferone had hardly any effect on **proteoglycan** synthesis, whereas xylosyl-beta-D-(4-methylumbelliferone) produced a large amount of glycosaminoglycan chains. The present results indicate that cells cultured with 4-methylumbelliferone produce a hyaluronic-acid-deficient extracellular matrix, which will be useful for functional studies of hyaluronic acid. (C) 1995 Academic Press, Inc.

L12 ANSWER 17 OF 42 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 95211779 MEDLINE
DOCUMENT NUMBER: 95211779 PubMed ID: 7697662
TITLE: Iduronic **acid**-rich proteoglycans (PGIdoA) and human post-burn scar maturation: isolation and characterization.
AUTHOR: Garg H G; Siebert J W; Garg A; Neame P J
CORPORATE SOURCE: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Shriners Burns Institute, Boston, MA.
SOURCE: CARBOHYDRATE RESEARCH, (1995 Feb 1) 267 (1) 105-13. Journal code: 0043535. ISSN: 0008-6215.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950510
Last Updated on STN: 19950510
Entered Medline: 19950504

AB **Proteoglycans** (PGs) were **extracted** from human hypertrophic and normal scar tissues from two different stages of maturation after burn injury, under dissociative conditions (4 M guanidinium chloride containing proteinase inhibitors). The extracts were fractionated by ion-exchange chromatography, followed by **ethanol** precipitation, to give PG-containing iduronic **acid** (PGIdoA). The size of the PGIdoA decreased with the maturation of scars. Glycosaminoglycan (GAG) chains from PGIdoA were released by alkaline borohydride treatment, and their M(r) values were evaluated by polyacrylamide gel electrophoresis. The M(r) values for PGIdoA protein cores of the hypertrophic scars (5+ years and 2-5 years) and normal scar (5+ years and 2-5 years) were 22.6, 25, 19 and 21 kDa, respectively. The iduronic **acid** content of PGIdoA from both types of scar increased in their maturation phase. The M(r) values of PGIdoA decreased with maturation. PGIdoA carried the sulfate group mainly attached at C-4 of the 2-amino-2-deoxy-D-galactose residue. The NH2-terminal amino **acid** sequences of all the PGIdoA were similar to those of normal human skin or bone PG II (decorin) (i.e., Asp-Glu-Ala-B-Gly-Ile-Gly-Pro-Glu-Val-Pro-Asp-Asp-Arg).

L12 ANSWER 18 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 94:557895 SCISEARCH
THE GENUINE ARTICLE: PF145
TITLE: A NOVEL OLIGOSACCHARIDE, XYLOSYL-BETA-1-4XYLOSYL-BETA-1-(4-METHYLUMBELLIFERONE), SYNTHESIZED BY CULTURED HUMAN SKIN FIBROBLASTS IN THE PRESENCE OF 4-METHYLUMBELLIFERYL-BETA-D-

XYLOSIDE
AUTHOR: IZUMI J; **TAKAGAKI K**; NAKAMURA T; SHIBATA S; KOJIMA K; KATO I; ENDO M (Reprint)
CORPORATE SOURCE: HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, HIROSAKI, AOMORI 036, JAPAN (Reprint); HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, HIROSAKI, AOMORI 036, JAPAN; RES INST GLYCOTECHNOL, HIROSAKI, AOMORI 036, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF BIOCHEMISTRY, (SEP 1994) Vol. 116, No. 3, pp. 524-529.
ISSN: 0021-924X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 23

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB 4-Methylumbelliferyl-beta-D-xyloside (Xyl-MU) was added to the medium of cultured human skin fibroblasts. After incubation, the culture medium was pooled, concentrated with a lyophilizer, and dialyzed against distilled water. Then the Xyl-MU derivatives in the diffusate were purified by gel-filtration and HPLC. A novel Xyl-MU derivative was obtained, in addition to the previously reported Xyl-MU derivatives, Xyl-MU-induced glycosaminoglycan (GAG-MU), SA-Gal-Xyl-MU, GlcA-Xyl-MU, Gal-Gal-Xyl-MU, and Gal-Xyl-MU. This Xyl-MU derivative was subjected to carbohydrate composition analysis, enzyme digestion, Smith degradation and ion-spray mass spectrometric analysis, and the results indicated that it was Xyl beta 1-4Xyl beta 1-MU, Although the quantity of Xyl beta 1-4Xyl beta 1-MU synthesized by human skin fibroblasts increased with incubation time, its production was independent of that of the GAG-MU. Xyl-Xyl-MU is different from the intermediates in the regular pathway of GAG-MU biosynthesis initiated by added Xyl-MU, posing an interesting question as to its significance in GAG biosynthesis.

L12 ANSWER 19 OF 42 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 94:693600 SCISEARCH
THE GENUINE ARTICLE: PN856
TITLE: A METHOD FOR DETERMINATION OF GALACTOSYLTRANSFERASE-I ACTIVITY SYNTHESIZING THE **PROTEOGLYCAN** LINKAGE REGION
AUTHOR: HIGUCHI T; TAMURA S (Reprint); **TAKAGAKI K**; NAKAMURA T; MORIKAWA A; TANAKA K; TANAKA A; SAITO Y; ENDO M
CORPORATE SOURCE: HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, 5 ZAIFU CHO, HIROSAKI, AOMORI 036, JAPAN (Reprint); HIROSAKI UNIV, SCH MED, DEPT BIOCHEM, HIROSAKI, AOMORI 036, JAPAN; HIROSAKI UNIV, SCH MED, DEPT OBSTET & GYNECOL, HIROSAKI, AOMORI 036, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS, (SEP 1994) Vol. 29, No. 2, pp. 135-142.
ISSN: 0165-022X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 18

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An assay method was devised for measuring the activity of galactosyltransferase I (UDP-D-galactose:D-xylose galactosyltransferase), which is one of the enzymes synthesizing the linkage region between the

core protein and glycosaminoglycan chains of **proteoglycan**. For this method, the reaction mixture contained a fluorescent substrate, 4-methylumbelliferyl-beta-D-xyloside as an acceptor, UDP-galactose as a donor and D-galactal as a competitive inhibitor of endogenous beta-galactosidase in the enzyme solution. The reaction mixture was incubated at 37 degrees C with enzyme solution prepared from an extract of cultured cells, and galactosyl-xylosyl-4-methylumbelliferone was produced as a reaction product. Measurement of galactosyltransferase I activity was performed by separation and quantitative analysis of this reaction product using high-performance liquid chromatography. Utilizing this method, easier and more sensitive detection of galactosyltransferase I activity in a cell-free system became possible. Application of the method revealed that cultured human skin fibroblasts contained galactosyltransferase I activity.

L12 ANSWER 20 OF 42 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6

ACCESSION NUMBER: 1993:415968 BIOSIS
DOCUMENT NUMBER: PREV199396081693
TITLE: Regulation of corneal collagen fibrillogenesis in vitro by corneal proteoglycan (lumican and decorin) core proteins.
AUTHOR(S): Rada, Jody A.; Cornuet, Pamela K.; Hassell, John R.
CORPORATE SOURCE: Eye and Ear Inst. Pittsburgh, Dep. Ophthalmol., Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15213 USA
SOURCE: Experimental Eye Research, (1993) Vol. 56, No. 6, pp. 635-648.
ISSN: 0014-4835.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Corneal transparency is dependent on the size and arrangement of collagen fibrils within the corneal stroma. The corneal stroma is composed primarily of collagen type I fibrils and two proteoglycans; one with chondroitin/dermatan sulfate side-chains (decorin) and one with keratan sulfate side-chains (lumican). We investigated the effects of the corneal proteoglycans on corneal collagen fibrillogenesis, utilizing an in vitro assay for fibril formation. Collagen was extracted from bovine corneal stromas with 0.1 M **acetic acid** and monomers purified by NaCl precipitation. Decorin and lumican were extracted from bovine corneal stroma with either 0.7 M NaCl or 4 M guanidine HCl and purified by DEAE and Sepharose CL-4B chromatography. Decorin and lumican from both extracts inhibited the rate of collagen fibrillogenesis and the development of turbidity in fibrillogenesis samples. Furthermore, the core proteins of decorin and lumican were shown to be as effective as the intact proteoglycans in inhibiting fibrillogenesis. The decorin core protein isolated from the 0.7 M NaCl extract was determined to be a 20 kDa fragment which lacks the C-terminal half of the core protein. This fragment was apprxeq 1/36 as effective in inhibiting fibrillogenesis as intact decorin isolated from guanidine extracts. This suggests that the C-terminal half of the decorin core plays an important role in the interaction of this **proteoglycan** with collagen. Lumican **extracted** with 0.7 M NaCl was slightly smaller and was only one-sixth as effective in inhibiting collagen fibril formation as 4 M guanidine extracted lumican. Furthermore reduction and alkylation of lumican core protein abolished the inhibitory activity of the core protein on collagen fibrillogenesis. Electron microscopic examination indicated that fibrils formed in the presence of lumican and lumican core protein were significantly thinner than fibrils formed in the absence of proteoglycans. The results of these studies indicate that in addition to decorin, lumican retards corneal collagen fibrillogenesis and results in

the formation of collagen fibrils which are significantly thinner than those formed in the absence of any proteoglycan. The inhibitory activity of lumican or decorin on collagen fibrillogenesis resides in the core proteins of these proteoglycans, not the glycosaminoglycan side chains, and that interaction of the lumican core protein with collagen appears to be dependent on the presence of disulfide bridges within the protein core.

L12 ANSWER 21 OF 42 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 94099623 MEDLINE
DOCUMENT NUMBER: 94099623 PubMed ID: 8274027
TITLE: Uronic **acid**-containing glycosaminoglycans and keratan sulfate are present in the tectorial membrane of the inner ear: functional implications.
AUTHOR: Thalmann I; Machiki K; Calabro A; Hascall V C; Thalmann R
CORPORATE SOURCE: Department of Otolaryngology, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: NIDCD DC 00384 (NIDCD)
NIDCD DC 01374 (NIDCD)
NIDCD DC 01414 (NIDCD)
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1993 Dec) 307 (2) 391-6.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940215
Last Updated on STN: 19940215
Entered Medline: 19940128

AB The tectorial membrane is a gel-like, acellular connective tissue overlying the microscopic organ of Corti--the auditory sensory structure. It is instrumental in the sound-synchronous deflection of the stereocilia of the hair cells, a central event in auditory transduction. It is well established that collagen, primarily type II, constitutes the major protein of the tectorial membrane, with smaller amounts of types IX and XI also present. However, conclusive information on the proteoglycans in this structure is lacking. Tectorial membranes were extracted with a 4 M guanidine--HCl solvent, and **proteoglycans isolated** after **ethanol** precipitation and collagenase treatment. A colorimetric assay based on the binding of the cationic dye safranin O to glycosaminoglycans, in combination with enzymatic techniques, detected significant amounts of chondroitin sulfate and keratan sulfate (0.29 and 0.17% on a wet weight basis, respectively). Agarose-polyacrylamide electrophoresis of chondroitinase-digested samples revealed a core protein with a similar molecular mass to that of the large **cartilage** proteoglycan aggrecan. This proteoglycan reacted with the antibody 3-B-3 (recognizing modified chondroitin 6-sulfate linkage region oligosaccharides). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed several low molecular mass proteins which reacted with 5-D-4, specific for keratan sulfate, one of which showed characteristics of fibromodulin. Comparison of the quantitative aspects of various connective tissue components of tectorial membrane with other type II collagen-containing structures revealed that this tissue resembles highly hydrated **cartilage**.

L12 ANSWER 22 OF 42 EMBASE
ACCESSION NUMBER: 92293602 EMBASE
DOCUMENT NUMBER: 1992293602

TITLE: A new endo-.beta.-galactosidase acting on the Gal.beta.1-3Gal linkage of the **proteoglycan** linkage region.
AUTHOR: **Takagaki K.**; Nakamura T.; Takeda Y.; Daidouji K.; Endo M.
CORPORATE SOURCE: Dept. of Biochemistry, Hirosaki University School of Med., 5 Zaifu-cho,Hirosaki 036, Japan
SOURCE: Journal of Biological Chemistry, (1992) 267/26 (18558-18563).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A new type of endo-.beta.-galactosidase acting on the linkage region of peptidochondroitin sulfate was isolated from the mid-gut gland of the mollusk Patinopecten. The purification procedure included ammonium sulfate precipitation, Sephacryl S-200HR gel filtration, DEAE-Sephacel chromatography, and TSKgel Phenyl-5PW RP high performance liquid chromatography. The purified enzyme was free from exoglycosidases, sulfatases, and phosphatases. The specificity of the enzyme was as follows. 1) It acted on the internal galactoside linkage of sugar chains; 2) it specifically hydrolyzed the galactosylgalactose (Gal.beta.1-3Gal) linkage, but not the galactosylxylose (Gal.beta.1-4Xyl) linkage in the linkage region of peptidoglycans; 3) the enzyme activity was unaffected by the type of glycosaminoglycan, chondroitin sulfate, dermatan sulfate or heparan sulfate used as a substrate; 4) keratan sulfate and some oligosaccharides from glycolipid were not degraded by the enzyme. These properties of the endo-.beta.- galactosidase characterize it as a new endo-.beta.-galactosidase with unique specificity.

L12 ANSWER 23 OF 42 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 90243714 MEDLINE
DOCUMENT NUMBER: 90243714 PubMed ID: 2139877
TITLE: Isoforms of corneal keratan sulfate proteoglycan.
AUTHOR: Funderburgh J L; Conrad G W
CORPORATE SOURCE: Division of Biology, Kansas State University, Manhattan 66506.
CONTRACT NUMBER: EY 00952 (NEI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1990 May 15) 265 (14) 8297-303.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199006
ENTRY DATE: Entered STN: 19900706
Last Updated on STN: 20000303
Entered Medline: 19900613

AB Bovine corneal keratan sulfate proteoglycan was found to contain three major protein components. Two proteins (37 and 25 kDa) were released from the proteoglycan by endo-beta-galactosidase, N-glycanase, or chemical deglycosylation. A smaller protein (20 kDa), not covalently linked to keratan sulfate, co-**purified** with the **proteoglycan** by conventional and high performance ion exchange chromatography, by **ethanol** precipitation, and by affinity purification on columns of monoclonal antibody to keratan sulfate, but could be separated from the

proteoglycan by gel filtration chromatography in dissociative agents. The three proteins produced different fragmentation patterns on sodium dodecyl sulfate-polyacrylamide gel electrophoresis after digestion with V8 protease, and each had unique two-dimensional tryptic peptide maps. The N-terminal amino **acid** sequence of the core proteins differed. In addition, the proteoglycans containing these proteins differed in molecular size, suggesting different levels of glycosylation of the two core proteins. Similarity of the core proteins was suggested by similar amino **acid** composition, similarities in tryptic maps, and antigenic cross-reactivity. Corneal keratan sulfate proteoglycan, therefore, seems to occur in two different, but related, forms whose core proteins may represent members of a homologous family.

L12 ANSWER 24 OF 42 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 91168191 MEDLINE
DOCUMENT NUMBER: 91168191 PubMed ID: 2076520
TITLE: Purification and characterization of iduronic **acid** -rich and glucuronic **acid**-rich proteoglycans implicated in human post-burn keloid scar.
AUTHOR: Garg H G; Lippay E W; Burd D A; Neame P J
CORPORATE SOURCE: Department of Biological Chemistry, and Molecular Pharmacology, Harvard Medical School, Shriners Burns Institute, Boston, Massachusetts.
SOURCE: CARBOHYDRATE RESEARCH, (1990 Oct 25) 207 (2) 295-305. Journal code: 0043535. ISSN: 0008-6215.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199104
ENTRY DATE: Entered STN: 19910512
Last Updated on STN: 20000303
Entered Medline: 19910424

AB Small **proteoglycans** (PGs), **extracted** from human keloid scar tissue with 4M guanidinium chloride and fractionated by DEAE-cellulose chromatography, were separated by **ethanol** precipitation into one L-iduronic **acid**-rich and one D-glucuronic **acid**-rich fraction. The size of the L-iduronic **acid** -rich PG was 102 kDa with a 27 kDa glycosaminoglycan chain, that of the D-glucuronic **acid**-rich PG was 90 kDa with a 26 kDa glycosaminoglycan chain, and the protein core of both PGs was 14.5 kDa. The two PGs carried sulfate groups mostly attached at C-4 of the 2-amino-2-deoxy-D-galactose units. The N-terminal amino **acid** sequence of both was similar to human bone PGII (decorin), normal and hypertrophic scar, and human dermal tissue PG.

L12 ANSWER 25 OF 42 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 10
ACCESSION NUMBER: 1989:475215 HCAPLUS
DOCUMENT NUMBER: 111:75215
TITLE: Small dermatan sulfate proteoglycans in human epidermis and dermis
AUTHOR(S): Garg, Hari G.; Burd, D. Andrew R.; Swann, David A.
CORPORATE SOURCE: Dep. Biol. Chem., Harvard Med. Sch., Boston, MA, 02114, USA
SOURCE: Biomedical Research (1989), 10(3), 197-207 CODEN: BRESD5; ISSN: 0388-6107
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Proteoglycans** were **isolated** from human epidermis and

Search completed by David Schreiber 308-4292

dermis by extn. with 4M guanidinium chloride, then fractionated on a DEAE-cellulose column. Pure dermatan sulfate proteoglycan (DSPG) was achieved by differential **ethanol** pptn. The Mr values of the epidermal and dermal DSPGs were 130 and 66 K, resp. Alk. borohydride treatment of DSPGs released glycosaminoglycan (GAG) chains and reduced xylose to xylitol, suggesting an O-glycoside linkage, i.e., a xylosyl-seryl linkage. The total uronic **acid** present in the DSPG was .apprx.90% iduronic **acid**. The av. Mr value of the GAG chains was 17.5 K, estd. by plotting the logs of the Mr of the stds. against their elution vols. on Sepharose CL-6B column and the no. of disaccharide units in the largest oligosaccharide obtained by hyaluronidase treatment of the corresponding proteoglycans. The GAG chains were polydisperse and carried sulfate residues predominantly attached at C-4 of galactosamine. SDS-PAGE anal. of the chondroitinase ABC-released epidermal DSPG protein core revealed a single protein core with Mr 45 K, whereas similar anal. of the chondroitinase ABC-released dermal DSPG protein core revealed 2 peptides, with Mr 21.5 and 14 K. The N-terminal amino **acid** sequence of the intact DSPGs had the following sequence: NH2-Asp-Glu-Ala-O-Gly-Ile-Gly-Pro-Glu-Val-Pro-Asp-Asp-Arg-Asp()-Phe-Glu-Pro-Ser()-Leu. Apparently, the human epidermis and dermis contain different populations of DSPGs.

L12 ANSWER 26 OF 42 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 89106645 MEDLINE
DOCUMENT NUMBER: 89106645 PubMed ID: 3215004
TITLE: Isolation and partial characterization of dermatan sulfate proteoglycans from human post-burn scar tissues.
AUTHOR: Swann D A; Garg H G; Hendry C J; Hermann H; Siebert E; Sotman S; Stafford W
CORPORATE SOURCE: Department of Surgery, Shriners Burns Institute, Boston, MA 02114.
SOURCE: COLLAGEN AND RELATED RESEARCH, (1988 Jul) 8 (4) 295-313. Journal code: 8102998. ISSN: 0174-173X.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198903
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19890302

AB Dermatan sulfate (DS) **proteoglycans** (PGs) were **extracted** from human post-burn scar (Sc) tissues with 4M guanidinium chloride and isolated from the extracts by DEAE-cellulose chromatography and by differential **ethanol** precipitation. The DS.PGs were further purified by Sepharose CL-6B column chromatography. The average molecular weight (Mr) of hypertrophic scar (HSc) tissue DS.PGs was 39,000 based on sedimentation equilibrium measurements. Alkaline borohydride treatment of DS.PGs liberated glycosaminoglycan (GAG) chains and the presence of xylitol indicated that these chains were attached to protein core by xylosyl residues. The average Mr of the DS.GAG chain from HSc and normal scar (NSc) samples were 23,500 and 20,000 respectively. After digestion of the HSc and NSc, DS.PGs with chondroitinase ABC in the presence of proteinase inhibitors, two peptide components with Mr values of 21,500 and 17,000 were detected by SDS-polyacrylamide gel electrophoresis using reducing conditions. Analysis of the protein core fractions derived from NSc and HSc DS.PGs by Sepharose CL-6B column chromatography showed the presence of a single NH2-terminal amino **acid** (aspartic **acid**) and also that the fractions with different KAV values had an

identical NH2-terminal sequence (A1-A5). The A1-A23 sequence of NSc DS.PG (major fraction, C): NH2Asp-Glu-Ala-O-Gly-Ile-Gly-Pro-Glu-Val-Pro-Asp-Asp-Arg-Asp-Phe-G lu-Pro- Ser-Leu-Gly-Pro-Val was the same as reported for a DS.PG isolated from human fetal membrane (HFM) tissue (Brennan et al., 1984). ELISA inhibition assay using monoclonal antibodies raised in rabbit against the NH2-terminal peptide (containing 15 amino **acids**) of human fetal membrane tissue were found to cross-react with HSc and NSc DS.PGs. Monoclonal antibodies to bovine skin DS.PGs protein core (Pearson et al., 1983) did not show any cross-reactivity with scar DS.PGs. These results show that the scar DS.PGs described here are different from normal bovine skin DS.PGs in the size and type of the protein core, and that in all the samples, the peptide components have the same NH2-terminal amino **acid** sequence.

L12 ANSWER 27 OF 42 MEDLINE
ACCESSION NUMBER: 88211176 MEDLINE
DOCUMENT NUMBER: 88211176 PubMed ID: 3130219
TITLE: Influence of cartilage proteoglycans on type II collagen fibrillogenesis.
AUTHOR: Kuijer R; van de Stadt R J; de Koning M H; van Kampen G P; van der Korst J K
CORPORATE SOURCE: Jan van Breemen Institute, Centre for Rheumatology and Rehabilitation, Amsterdam, The Netherlands.
SOURCE: CONNECTIVE TISSUE RESEARCH, (1988) 17 (2) 83-97.
Journal code: 0365263. ISSN: 0300-8207.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198806
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880620

AB The effects of various **proteoglycan** samples, **isolated** from human articular cartilage of different ages, on the rate of the lateral growth phase of the fibril formation of collagen type II were studied by turbidimetry. In general, proteoglycan aggregates accelerate fibrillogenesis, whereas non-aggregating proteoglycans retard this process. The only exception were non-aggregating proteoglycans from very young cartilage, which stimulated the fibril formation strongly. The extent of stimulation by proteoglycans from hip and knee cartilage were compared. The effects of non-aggregating proteoglycans dominate those of aggregated proteoglycans. Chondroitinase ABC digestion of proteoglycan samples did not change the effects on the fibrillogenesis of collagen type II, when these samples were isolated from 18 years-old knee cartilage. The collagen fibril formation was less stimulated in the presence of ABC-ase digested proteoglycan samples from 0-3 month-old knee cartilage, suggesting a primary role for keratan sulphate and a possible influence of chondroitin sulphate when keratan sulphate is not present. Only proteoglycans from very old cartilage were able to reduce the amount of collagen fibrils formed in vitro. Proteoglycans could not be detected bound to the fibril pellet despite the fact that part of the pellet was not dissolvable in **acetic acid**. It is concluded that proteoglycans may play a regulatory role in collagen type II fibril formation in articular cartilage.

L12 ANSWER 28 OF 42 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 12
ACCESSION NUMBER: 1987:172949 HCAPLUS
DOCUMENT NUMBER: 106:172949

Search completed by David Schreiber 308-4292

TITLE: An L-arabino-D-galactan and an L-arabino-D-galactan-containing proteoglycan from radish (Raphanus sativus) seeds
AUTHOR(S): Tsumuraya, Yoichi; Hashimoto, Yohichi; Yamamoto, Shiguru
CORPORATE SOURCE: Fac. Sci., Saitama Univ., Urawa, 338, Japan
SOURCE: Carbohydrate Research (1987), 161(1), 113-26
CODEN: CRBRAT; ISSN: 0008-6215
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An L-arabino-D-galactan and an L-arabino-D-galactan-contg. **proteoglycan** were **isolated** from hot phosphate-buffered saline exts. of radish seeds by **ethanol** fractionation, ion-exchange chromatog., and gel filtration, and found homogeneous by ultracentrifuge anal. and high-voltage electrophoresis. The proteoglycan consisted of 86% of a polysaccharide component contg. L-arabinose and D-galactose as major sugar constituents, together with small proportions of D-xylose, D-glucose, and uronic **acids**, and 9% of a hydroxyproline-contg. protein. Methylation anal., periodate oxidn., and enzymic degrdns. indicated a backbone chain of (1.fwdarw.6)-linked .beta.-D-galactosyl residues and uronosyl groups. The .alpha.-L-arabinofuranosyl residues were located mainly in the outer regions as nonreducing groups, as well as O-2 or 5-linked inner chain residues, and O-2,5- or -3,5-linked branching residues. Reductive, alk. degrdn. of the proteoglycan indicated that the polysaccharide chains were partly linked through O-glycosyl linkages to the threonine residues of the polypeptide chains. The proteoglycans from radish leaves and seeds appeared to share common antigenic determinant(s). The radish-seed arabinogalactan had a high content (81%) of L-arabinose and its basic structure seemed to be similar to that of the polysaccharide component of the proteoglycan.

L12 ANSWER 29 OF 42 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 85031874 EMBASE
DOCUMENT NUMBER: 1985031874
TITLE: Pericellular coat of chick embryo chondrocytes: Structural role of hyaluronate.
AUTHOR: Goldberg R.L.; Toole B.P.
CORPORATE SOURCE: Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA 02111, United States
SOURCE: Journal of Cell Biology, (1984) 99/6 (2114-2122).
CODEN: JCLBA3
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 031 Arthritis and Rheumatism
023 Nuclear Medicine
021 Developmental Biology and Teratology
LANGUAGE: English

AB Chondrocytes produce large pericellular coats in vitro that can be visualized by the exclusion of particles, e.g., fixed erythrocytes, and that are removed by treatment with Streptomyces hyaluronidase, which is specific for hyaluronate. In this study, we examined the kinetics of formation of these coats and the relationship of hyaluronate and **proteoglycan** to coat structure. Chondrocytes were **isolated** from chick tibia cartilage by collagenase-trypsin digestion and were characterized by their morphology and by their synthesis of both type II collagen and high molecular weight proteoglycans. The degree of spreading of the chondrocytes and the size of the coats were quantitated at various

times subsequent to seeding by tracing phase-contrast photomicrographs of the cultures. After seeding, the chondrocytes attached themselves to the tissue culture dish and exhibited coats within 4 h. The coats reached a maximum size after 3-4 d and subsequently decreased over the next 2-3 d. Subcultured chondrocytes produced a large coat only if passaged before 4 d. Both primary and first passage cells, with or without coats, produced type II collagen but not type I collagen as determined by enzyme-linked immunosorbent assay. Treatment with Streptomyces hyaluronidase (1.0 mU/ml, 15 min), which completely removed the coat, released 58% of the chondroitin sulfate but only 9% of the proteins associated with the cell surface. The proteins released by hyaluronidase were not digestible by bacterial collagenase. Monensin and cycloheximide (0.01-10 .mu.M, 48 h) caused a dose-dependent decrease in coat size that was linearly correlated to synthesis of cell surface hyaluronate (r = 0,98) but not chondroitin sulfate (r = 0.2). We conclude that the coat surrounding chondrocytes is dependent on hyaluronate for its structure and that hyaluronate retains a large proportion of the proteoglycan in the coat.

L12 ANSWER 30 OF 42 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 84111642 MEDLINE
DOCUMENT NUMBER: 84111642 PubMed ID: 6420413
TITLE: Phosphorylation of chondroitin sulfate in proteoglycans from the swarm rat chondrosarcoma.
AUTHOR: Oegema T R Jr; Kraft E L; Jourdian G W; Van Valen T R
CONTRACT NUMBER: AM32372 (NIADDK)
CA22558 (NCI)
HL21612 (NHLBI)
+
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1984 Feb 10) 259 (3) 1720-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198403
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19840316

AB **Proteoglycans isolated** from the Swarm rat chondrosarcoma were shown to contain 35 mol of phosphate/mol of proteoglycan. While 20% of this phosphate was released by digestion with dilute alkali in the presence of sodium borohydride and is presumably of the phosphoserine/phosphothreonine type, 78% of the phosphate copurified with the peptide-free chondroitin sulfate chains. When chondroitin sulfate chains purified by **ethanol** precipitation or Sephacryl S200 column chromatography were digested with chondroitinase AC and the digests chromatographed on Bio-Gel P-4, the phosphate co-migrated with a carbohydrate fragment that contained 2 glucuronic **acid** (one as delta 4,5-unsaturated sugar), 1-galactosamine, 2-galactose, and 1-phosphate residue/xylitol. A second fragment of similar composition but lacking phosphate was also recovered in a ratio of about 3 to 1 relative to the phosphorylated fragment. The phosphate in the chondroitin sulfate linkage region fragment had the alkaline phosphatase sensitivity as well as 31P NMR spectra of a monophosphate esterified to a secondary sugar alcohol. The phosphate was localized on the C-2 of the chain initiating xylose since these residues as xylitol showed a delayed release during **acid** hydrolysis and the xylitol was recovered intact after periodate oxidation. In the chondrosarcoma, 2-phosphoxylose appears to be

a normal synthetic product since [32P]phosphate was readily incorporated into the proteoglycan and the incorporated isotope had similar biochemical properties as the unlabeled phosphate.

L12 ANSWER 31 OF 42 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 84121506 EMBASE
DOCUMENT NUMBER: 1984121506
TITLE: Phosphorylation of chondroitin sulfate in proteoglycans from the swarm rat chondrosarcoma.
AUTHOR: Oegema Jr. T.R.; Kraft E.L.; Jourdian G.W.; Van Valen T.R.
CORPORATE SOURCE: Department of Orthopaedic Surgery, University of Minnesota, Minneapolis, MN 55455, United States
SOURCE: Journal of Biological Chemistry, (1984) 259/3 (1720-1726). CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
033 Orthopedic Surgery
016 Cancer
LANGUAGE: English
AB **Proteoglycans isolated** from the Swarm rat chondrosarcoma were shown to contain 35 mol of phosphate/mol of proteoglycan. While 20% of this phosphate was released by digestion with dilute alkali in the presence of sodium borohydride and is presumably of the phosphoserine/phosphothreonine type, 78% of the phosphate copurified with the peptide-free chondroitin sulfate chains. When chondroitin sulfate chains purified by **ethanol** precipitation or Sephacryl S200 column chromatography were digested with chondroitinase AC and the digests chromatographed on Bio-Gel P-4, the phosphate co-migrated with a carbohydrate fragment that contained 2 glucuronic **acid** (one as .DELTA.4,5-unsaturated sugar), 1-galactosamine, 2-galactose, and 1-phosphate residue/xylitol. A second fragment of similar composition but lacking phosphate was also recovered in a ratio of about 3 to 1 relative to the phosphorylated fragment. The phosphate in the chondroitin sulfate linkage region fragment had the alkaline phosphatase sensitivity as well as 31P NMR spectra of a monophosphate esterified to a secondary sugar alcohol. The phosphate was localized on the C-2 of the chain initiating xylose since these residues as xylitol showed a delayed release during **acid** hydrolysis and the xylitol was recovered intact after periodate oxidation. In the chondrosarcoma, 2-phosphoxylose appears to be a normal synthetic produce since [32P]phosphate was readily incorporated into the proteoglycan and the incorporated isotope had similar biochemical properties as the unlabeled phosphate.

L12 ANSWER 32 OF 42 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1984:344272 BIOSIS
DOCUMENT NUMBER: BA78:80752
TITLE: SELECTIVE DE MINERALIZATION OF HARD TISSUES IN ORGANIC SOLVENTS RETENTION OR EXTRACTION OF PROTEO GLYCAN.
AUTHOR(S): SCOTT J E; BURTON S M
CORPORATE SOURCE: CHEM. MORPHOLOGY, CHEM. BUILD., UNIV. MANCHESTER, BRUNSWICK ST., MANCHESTER M13 9PW.
SOURCE: J MICROSC (OXF), (1984) 134 (3), 291-298. CODEN: JMICAR. ISSN: 0022-2720.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Ideally, demineralization of tissues, fossils, etc., should not result in removal of other chemical species along with the mineral components. Organic solvents containing alkylammonium salts of EDTA are good

Search completed by David Schreiber 308-4292

demineralizing media, offering great flexibility and a variety of solvent properties which can be chosen either to extract or to retain tissue polymers in situ. The properties of the alkylammonium cation are crucial in determining whether a given solvent will extract tissue polyanions (i.e. nucleic **acids**, proteoglycans, **acid** glycoproteins, etc.) or not. The situation is analyzed for the general case, using the fact that a simple ion-exchange reaction determines the outcome. Some solvent-alkylammonium combinations, e.g. **ethanol** -trimethylammonium, are very poor solvents for proteoglycans, leaving them behind in the tissue; by increasing the size of the organic portion of the alkylammonium salt (e.g. to tri-n-butylammonium), or changing the solvents to, e.g., dimethyl sulfoxide, the **proteoglycan** can be **extracted** from **cartilage**, etc. The anionic half of the alkylammonium salt plays a relatively minor role. The exception is EDTA itself, which sequestrates inorganic ions (e.g., Na+) in organic solvents, thus driving the ion-exchange towards completion, with efficient conversion to alkylammonium salts of the polyanion. These results, and the chemical background to them, constitute a general framework within which various strategies for demineralization (with or without **extraction** of tissue polyanion such as **proteoglycans**) can be formulated.

L12 ANSWER 33 OF 42 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 84008151 MEDLINE
DOCUMENT NUMBER: 84008151 PubMed ID: 6619129
TITLE: Biosynthesis of O-linked oligosaccharides on proteoglycans by chondrocytes from the swarm rat chondrosarcoma.
AUTHOR: Thonar E J; Lohmander L S; Kimura J H; Fellini S A; Yanagishita M; Hascall V C
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1983 Oct 10) 258 (19) 11564-70.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198311
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19831123

AB The core protein of proteoglycans from **cartilage** is substituted with glycosaminoglycans as well as N- and O-glycosidically linked oligosaccharides. We have taken advantage of the long intracellular half-life of the core protein precursor to the rat chondrosarcoma proteoglycan to study the temporal relationship between the addition of the chondroitin sulfate chains and the O-linked oligosaccharides onto the core protein during the formation of the completed proteoglycan molecule. Chondrocyte cultures were pulsed on day 2 with [6-3H]glucosamine for times ranging from 30-420 min. Media and corresponding 4% zwittergent, 4 M guanidine HCl extracts were then pooled and subjected to dissociative density gradient ultracentrifugation to yield **purified** **proteoglycan** monomers which were then subjected to alkaline borohydride treatment. The released chondroitin sulfate chains were then purified by precipitation with 50% (v/v) **ethanol**. The O-linked oligosaccharide-alditols in the supernatant fractions were purified by molecular sieve chromatography on Bio-Gel P-6, and analyzed after digestion with alpha-neuraminidase and subsequent chromatography on Bio-Gel P-2. The different O-linked oligosaccharide-alditols were identified from their hexosamine and hexosaminitol contents. The kinetics

of entry of 3H label into N-acetylgalactosamine of chondroitin sulfate was indistinguishable from that into either N-acetylglucosamine or N-acetylgalactosaminitol residues of the oligosaccharide-alditols, with half-times to linear incorporation of 10-17 min. These results show that initiation as well as completion of the O-linked oligosaccharides on the core protein occurs essentially at the same time that chondroitin sulfate chains are added. The results suggest that these biosynthetic processes occur in the Golgi apparatus during the last few minutes of the total intracellular dwell time (half-time of about 90 min) of the core protein acceptor.

L12 ANSWER 34 OF 42 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1984:176624 BIOSIS
DOCUMENT NUMBER: BA77:9608
TITLE: ISOLATION AND CHARACTERIZATION OF 3RD PROTEO GLYCAN PG-LT FROM CHICK EMBRYO **CARTILAGE** WHICH CONTAINS DI SULFIDE BONDED COLLAGENOUS POLY PEPTIDE.
AUTHOR(S): NORO A; KIMATA K; OIKE Y; SHINOMURA T; MAEDA N; YANO S; TAKAHASHI N; SUZUKI S
CORPORATE SOURCE: DEP. CHEM., FAC. SCI., NAGOYA UNIV., NAGOYA 464, JPN.
SOURCE: J BIOL CHEM, (1983) 258 (15), 9323-9331.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Chick embryo epiphyseal **cartilage** contains 3 different proteoglycan species (PG-H, PG-Lb, and PG-Lt). The **purification** and characterization of the 3rd **proteoglycan**, PG-Lt, was investigated. The proteoglycan can be separated from the other 2 by virtue of its low buoyant density in a CsCl density gradient and further purified by consecutive ion exchange and gel chromatography. The final preparation is composed of PG-Lt monomer and PG-Lt oligomer. The amino **acid** composition of PG-Lt is quite different from that of PG-H and PG-Lb and rather resembles that of collagens with respect to high content of glycine and high degrees of hydroxylation of proline and lysine. PG-Lt monomer is composed of disulfide-bonded subunits of MW .simeq. 120,000 and 190,000 as demonstrated by its gel electrophoretic behavior after reduction with 2-mercaptoethanol. The latter, but not the former, contains dermatan sulfate chains with glucuronic **acid**/iduronic **acid** residues and yields a protein-enriched core molecule of MW .simeq. 100,000 after digestion with chondroitinase ABC. Both of the protein subunits are completely digestible with bacterial collagenase. Immunofluorescence microscopic examination of **cartilage** tissues, using an antibody against PG-Lt, shows that this proteoglycan exists in both the **cartilage** matrix and perichondrial noncartilagenous region. When chondrocytes are plated onto tissue culture dishes, the antibody stains strands found on the cell surfaces and in the intercellular space of substrate-attached cell layers, suggesting that PG-Lt mediates cell-to-cell and cell-to-substrate contacts.

L12 ANSWER 35 OF 42 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1984:506889 HCAPLUS
DOCUMENT NUMBER: 101:106889
TITLE: Selective demineralization of hard tissues in organic solvents: retention or **extraction** of **proteoglycan**?
AUTHOR(S): Scott, J. E.; Burton, Shirley M.
CORPORATE SOURCE: Univ. Manchester, Manchester, M13 9PW, UK
SOURCE: Journal of Microscopy (Oxford, United Kingdom) (1983), 134(3), 291-7

Search completed by David Schreiber 308-4292

CODEN: JMICAR; ISSN: 0022-2720
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Ideally, demineralization of tissues, fossils, etc., should not result in removal of other chem. species along with the mineral components. Org. solvents contg. alkylammonium salts of EDTA are good demineralizing media (Scott, J. E.; Kyffin, T. W., 1978), offering great flexibility and a variety of solvent properties which can be chosen either to ext. or to retain tissue polymers in situ. The properties of the alkylammonium cation are crucial in detg. whether a given solvent will ext. tissue polyanions (i.e., nucleic **acids**, proteoglycans, **acid** glycoproteins, etc.) or not. The situation is analyzed for the general case, by using the fact that a simple ion-exchange reaction dets. the outcome. Some solvent-alkylammonium combinations, e.g. EtOH-trimethylammonium, are very poor solvents for proteoglycans, leaving them behind in the tissue, whereas by increasing the size of the org. portion of the alkylammonium salt (e.g., to tri-n-butylammonium) or changing the solvents to e.g., DMSO, the proteoglycan can be extd. from **cartilage**, etc. The anionic half of the alkylammonium salt plays a relatively minor role. The exception is EDTA itself, which sequestrates inorg. ions (e.g. Na+) in org. solvents, thus driving the ion-exchange towards completion, with efficient conversion to alkylammonium salts of the polyanion. These results, and the chem. background to them, constitute a general framework within which various strategies for demineralization (with or without extn. of tissue polyanion such as proteoglycans) can be formulated.

L12 ANSWER 36 OF 42 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 83101274 MEDLINE
DOCUMENT NUMBER: 83101274 PubMed ID: 6217835
TITLE: Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma.
AUTHOR: Kleinman H K; McGarvey M L; Liotta L A; Robey P G; Tryggvason K; Martin G R
SOURCE: BIOCHEMISTRY, (1982 Nov 23) 21 (24) 6188-93.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198303
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19980206
Entered Medline: 19830311

AB We have studied the extractability of type IV collagen, laminin, and heparan sulfate proteoglycan from EHS tumor tissue growth in normal and lathyritic animals. Laminin and heparan sulfate **proteoglycan** were readily **extracted** with chaotropic solvents from both normal and lathyritic tissue. The collagenous component was only solubilized from lathyritic tissue in the presence of a reducing agent. These results indicate that lysine-derived cross-links and disulfide bonds stabilize the collagenous component in the matrix but not the laminin or the heparan sulfate proteoglycan. The majority of the collagen present in the extracts had a native triple helix based upon the pattern of peptides resistant to pepsin digestion and visualization in the electron microscope by the rotary shadow technique. This protein was composed of chains (Mr 185000 and 170000) identical in migration to the chains of newly synthesized type IV procollagen. This finding confirms earlier work that

indicates that the biosynthetic form, type IV procollagen, is incorporated as such in the basement membrane matrix. Material with smaller chains (Mr 160000 and 140000) appeared on storage in **acetic acid** solutions. These results indicate that the lower molecular weight collagen in acid extracts of basement membrane arises artifactually due to an endogenous acid-active protease.

L12 ANSWER 37 OF 42 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 81093128 EMBASE
DOCUMENT NUMBER: 1981093128
TITLE: Effects of anti-inflammatory drugs on proteoglycan degradation as studied in rabbit articular cartilage in organ culture.
AUTHOR: Comper W.D.; De Witt M.; Lowther D.A.
CORPORATE SOURCE: Dept. Biochem., Monash Univ., Clayton, Victoria 3168, Australia
SOURCE: Biochemical Pharmacology, (1981) 30/5 (459-468).
CODEN: BCPCA6
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal
FILE SEGMENT: 037 Drug Literature Index
030 Pharmacology
029 Clinical Biochemistry
033 Orthopedic Surgery
031 Arthritis and Rheumatism
LANGUAGE: English

AB The effects of anti-inflammatory drugs and other agents on in vitro degradation of cartilage proteoglycan of rabbit articular cartilage in organ culture were measured by 3 techniques: (1) the release of 35S-labeled proteoglycan from pre-labeled rabbit articular cartilage into organ culture media, (2) the distribution of radioactivity of such released material on Sepharose 2BCL columns under associative conditions, and (3) the elution profiles of the associated and dissociated forms of 3H-labeled **proteoglycans** dissociatively **extracted** from tissue after various times of incubation with 3H-acetate. The anti-inflammatory drugs studied were salicylate (0.5 and 3 mM), hydrocortisone (0.2 mM), indomethacin (0.2 and 1.0 mM), phenylbutazone (0.2 and 1.0 mM), D-penicillamine (0.2 and 1.0 mM), and colchicine (1 mM). These drugs, with the exception of D-penicillamine, inhibited proteoglycan degradation to varying degrees, depending on drug concentration. Inhibition of proteoglycan degradation, as measured by the quantity of released material from pre-labeled tissue, was paralleled by the inhibition of the breakdown of aggregates to smaller units that were included on Sepharose 2BCL. The autocatalytic degradation of proteoglycans in tissue culture and its inhibition by salicylate (3 mM) were also demonstrated in analysis of [3H]proteoglycans synthesized in vitro. These studies did not distinguish between inhibitory activities of the anti-inflammatory drugs at the levels of enzyme production and enzyme activity. Significant inhibitory activity, however, was recorded for cycloheximide (0.35 mM), puromycin (0.1 mM), and the antimicrotubular agent colchicine (1.0 mM), which suggests that activation of proteoglycan degradative processes in organ culture requires de novo synthesis of protein and associated transport mechanics.

L12 ANSWER 38 OF 42 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 82113014 MEDLINE
DOCUMENT NUMBER: 82113014 PubMed ID: 6798960
TITLE: Bovine aortic chondroitin sulphate- and dermatan sulphate-containing **proteoglycans**.

Search completed by David Schreiber 308-4292

Isolation, fractionatio
characterization.
AUTHOR: Kapoor R; Phelps C F; C nsson L A
SOURCE: BIOCHEMICAL JOURNAL, (1 197 (2) 259-68.
Journal code: 2984726R. 4-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOUR CLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198203
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19980206
Entered Medline: 19820313

AB 1. Guanidinium chloride (4M) in the presence of proteinase inhibitors extracted 90% of bovine aorta galactosaminoglycans as **proteoglycans** that were subsequently **purified** by ion-exchange and gel chromatography. 2. Fractionation of the calcium salts of the **purified proteoglycans** with increasing concentration of **ethanol** yielded fractions PG-25 (28%), PG-35 (45%) and PG-50 (37%). 3. Fraction PG-50 contained proteochondroitin 6-sulphate, whereas fractions PG-25 and PG-35 were proteodermatan sulphates of greatly different carbohydrate composition; the molar proportions of L-iduronate-N-acetylgalactosamine 4-sulphate, D-glucuronate-N-acetyl-galactosamine 4-sulphate and D-glucuronate-N-acetylgalactosamine 6-sulphate were 75: 18 :7 in fraction PG-25 and 14 :46 :40 in fraction PG-35. 4. The presence of alternating or mixed sequences with L-iduronate- and D-glucuronate-containing repeating disaccharides was indicated by the formation of tetrasaccharides after chondroitinase AC digestion (single L-iduronate residues) and by the release of fragments containing four or five consecutive D-glucuronate-N-acetylgalactosamine repeats after periodate oxidation and alkaline elimination. 5. The amino **acid** compositions of fractions PG-25 and PG-35 were similar and markedly different from that of fraction PG-50, which also contained more side chains.

L12 ANSWER 39 OF 42 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1979:178649 BIOSIS
DOCUMENT NUMBER: BA67:58649
TITLE: A RADIOASSAY FOR PROTEOLYTIC CLEAVAGE OF ISOLATED CARTILAGE PROTEO GLYCAN PART 1 SEPARATION PRINCIPLES AND VARIABLES.
AUTHOR(S): STEPHENS R W; PEMBERTON L I; GHOSH P; TAYLOR T K F
CORPORATE SOURCE: RAYMOND PURVES RES. LAB., UNIV. SYD., R. NORTH SHORE HOSP. SYD., ST. LEONARDS, N.S.W. 2065, AUST.
SOURCE: ANAL BIOCHEM, (1978) 90 (2), 726-736.
CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB A new micro-scale radioisotope technique is reported which enables the quantitation of chondroitin sulfate-peptides produced by the action of proteases on chick embryo **isolated proteoglycan**. Separation of digestion products from unchanged proteoglycan was achieved by selective trichloroacetic acid dissolution of their cetylpyridinium complexes held in glass fiber disks. Variables affecting this dissolution were trichloroacetic acid concentration and washing time, and the extent of proteoglycan degradation. Chymotryptic digests were fractionated on Sepharose 6B to obtain chondroitin sulfate-peptide clusters of varying sizes. Even large clusters of these glycosaminoglycan chains can be measured by the trichloroacetic acid dissolution of their cetylpyridinium complexes.

Search completed by David Schreiber 308-4292

L12 ANSWER 40 OF 42 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 77104730 EMBASE
DOCUMENT NUMBER: 1977104730
TITLE: Stimulation of proteoglycan synthesis in chick embryo sternum by serum and L 3,5,3' triiodothyronine.
AUTHOR: Audhya T.K.; Segen B.J.; Gibson K.D.
CORPORATE SOURCE: Roche Inst. Molec. Biol., Nutley, N.J. 07110, United States
SOURCE: Journal of Biological Chemistry, (1976) 251/12 (3763-3767). CODEN: JBCHA3
DOCUMENT TYPE: Journal
FILE SEGMENT: 037 Drug Literature Index
029 Clinical Biochemistry
001 Anatomy, Anthropology, Embryology and Histology
021 Developmental Biology and Teratology
003 Endocrinology
LANGUAGE: English

AB Incorporation of sulfate into alcian blue precipitable glycosaminoglycans of 12 day old chick embryo sterna is stimulated by addition, separately or together, of normal human serum and physiological concentrations of thyroid hormones (Audhya, T.K., and Gibson, K.D. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 604-608). The authors present evidence that this stimulation is due to increased synthesis of at least one proteoglycan, with minor alterations in the size and chemical composition of the glycosaminoglycans. Pulse chase experiments showed no detectable loss of label during the chase, in control sterna or sterna incubated with serum and L 3,5,3' triiodothyronine; thus, all incorporation was the result of synthesis of glycosaminoglycans. In double label experiments, with 35SO42- and [3H] glucose or [3H] acetate, the molar ratio of 3H and 35S incorporated into glycosaminoglycans was changed little, if at all, by addition of serum or triiodothyronine or both, at concentrations which increased incorporation up to 2 fold. Glycosaminoglycans isolated from these and other incubations gave similar elution patterns from agarose columns, and identical electrophoretic patterns on cellulose acetate. Digestion with chondroitinase ABC (chondroitin ABC lyase; EC 4.2.2.4.) showed that incorporation was into chondroitin sulfate and possibly hyaluronic acid, and that the proportions of non sulfated, 4 sulfated, and 6 sulfated disaccharide units differed little between stimulated and unstimulated sterna. Incorporation of [3H] serine into glycosaminoglycans from papain digests of sterna paralleled incorporation of 35SO42-, and indicated a number average molecular weight between 21,000 and 25,000 for the newly synthesized chondroitin sulfate. This value was confirmed by gel filtration chromatography, which also showed that the average molecular weight of the newly synthesized chondroitin sulfate decreased up to 15% under conditions of 2 fold stimulation. **Proteoglycans** were **extracted** from sterna incubated with [3H] serine and 35SO42- and analyzed by isopycnic centrifugation in CsCl and by zone sedimentation in a sucrose gradient. A major proteoglycan fraction could be separated by either method. Incorporation of both isotopes into this **proteoglycan** fraction, and into glycosaminoglycans **isolated** after papain digestion, was stimulated in a coordinate manner. Almost identical results were obtained with both separation techniques. The results indicate that the synthesis of the major proteoglycan, and probably also of a minor one, is stimulated by serum and triiodothyronine.

L12 ANSWER 41 OF 42 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 75224632 MEDLINE
DOCUMENT NUMBER: 75224632 PubMed ID: 125581

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Kam 09/916,250

TITLE: Fractionation of proteoglycans from bovine corneal stroma.
AUTHOR: Axelsson I; Heinegard D
SOURCE: BIOCHEMICAL JOURNAL, (1975 Mar) 145 (3) 491-500.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197511
ENTRY DATE: Entered STN: 19900310
Last Updated on STN: 19900310
Entered Medline: 19751106

AB **Proteoglycans** were **extracted** from bovine corneal stroma with 4M-guanidinum chloride, purified by DEAE-dellulose chromatography (Antonopoulos et al., 1974) and fractionated by precipitation with **ethanol** into three fractions of approximately equal weight. One of these fractions consisted of a proteoglycan that contained keratan sulphate as the only glycosaminoglycan. In the othertwo fractions proteoglycans that contained chondroitin sulphate, dermatan sulphate and keratan sulphate were present. Proteoglycans which had a more than tenfold excess of galactosaminoglycans over keratan sulphate could be obtianed by further subfractionation. The gel-chromatographic patterns of the glucosaminoglycans before and after digestion with chondroitinase AC differed for the fractions. The individual chondroitin sulphate chains seemed to be larger in cornea than in **cartilage**. Oligosaccharides, possibly covalently linked to the protein core of the **proteoglycans**, could be **isolated** from all fractions. The corneal proteoglycans were shown to have higher protein contents and to be of smaller molecular size than **cartilage** proteoglycans.

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ACCESSION NUMBER: 75133322 EMBASE
DOCUMENT NUMBER: 1975133322
TITLE: The **isolation** of soluble proteins, glycoproteins, and **proteoglycans** from bone.
AUTHOR: Herring G.M.; Ashton B.A.; Chipperfield A.R.
CORPORATE SOURCE: MRC External Sci. Staff, Bone Res. Lab., Churchill Hosp., Oxford, United Kingdom
SOURCE: Preparative Biochemistry, (1974) 4/2 (179-200).
CODEN: PRBCBQ
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
031 Arthritis and Rheumatism
LANGUAGE: English

AB Procedures are described for the isolation from bone of fractions containing proteins, glycoproteins and **proteoglycans**. **Extraction** of powdered bone with solutions of the sodium salts of ethylene diaminetetra **acetic acid** (EDTA) at pH 7.5 solubilized about 7% of the organic material. These extracts contained about 1.8% of the total collagen and at least 60% of the total noncollagenous protein of bone. The extracts were dialyzed against water to remove EDTA and then against a pH 5 buffer. At this stage a precipitate (G1) formed which was removed by centrifugation. The supernatant was applied to a column of the carboxylic ion exchange resin, Amberlite CG 50. The effluent at pH 5 contained the proteoglycans and more acidic glycoproteins and was therefore named the Acidic Fraction (AF). The material adsorbed to the resin (Fraction G2) was eluted by equilibration to pH 8. AF was further fractionated by cetylpyridinium chloride (CPC) precipitation into three relatively pure components: CP S, a glycoprotein

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soluble in CPC, bone sialoprotein which formed a CPC precipitate soluble in 0.2M $MgCl_2$; and a proteoglycan fraction which formed a CPC precipitate insoluble in 0.2M $MgCl_2$. The G2 fraction contained most of the soluble collagen together with glycoproteins and other noncollagenous proteins. These were fractionated by chromatography on DEAE cellulose at pH 7.2 using stepwise elution with increasing concentrations of NaCl. Some resolution of the mixture was obtained, though most of the fractions contained more than one component. These procedures were used on an analytical scale to assess the yields and recoveries of total protein, hydroxyproline and sialic acid in the fractions described above. This was compared with the large scale procedure for the preparation of the fractions, which were studied in previous work.